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PATENT
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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants: Coppens et al.

Serial No.: 08/898,736

Filed: July 23, 1997

Title: PROCESS FOR THE PREPARATION OF MALTED CEREALS

Group Art Unit: 1761

Examiner: C. Sherrer

SUPPLEMENTAL DECLARATION OF THEO COPPENS UNDER 37 CFR 1.132

Honorable Commissioner of Patents
and Trademarks
Washington, D.C. 20231

Dear Sir:

I, Theo Coppens, pursuant to 37 C.F.R. §1.132, declare as follows:

1. I am one of the inventors for the above-identified patent application.

2. In 1999, I asked Prof. C. Michiels, Professor of the Faculty of Agricultural and Applied Biological Sciences at Katholieke Universiteit Leuven in Belgium, to conduct the following experiments under my supervision to determine whether the medium and growth conditions described in Gyllang *et al.*

would provide activated spores. Those experiments and their results were first reported in my Declaration signed on July 9, 1999, and a Supplemental Declaration signed on March 3, 2000. A more detailed explanation of those experiments and their results is presented herewith.

Materials and Methods

3. Chemicals and Media. Peptone, Yeast Extract and Potato Dextrose Agar (PDA) were obtained from Unipath (Hampshire, United Kingdom). Dextrose was obtained from Merck-Belgolabo (Leuven, Belgium). Peptone, yeast extract and dextrose medium were prepared according to Kaiser et al. (1994). Peptone (2% w/v), yeast extract (1% w/v) and dextrose (2% w/v) were dissolved in deionised water. The medium was sterilized at 121°C for 15 minutes. The pH of the obtained medium was 6.4.

4. Fungal Strains: Cultivation and Preparation of Culture Homogenate. The strains *Rhizopus oryzae* ATCC 9363, *Aspergillus fumigatus* CBS 148.89 and Aspergillus *amstelodami* VTTD-76035 were obtained from respectively the American Type Culture Collection (ATCC, Manassas, VA, USA), Centraalbureau voor Schimmelcultures (CBS, Baarm, The Netherlands) and VTT (Technical Research Centre of Finland, Espoo, Finland) culture collections. The strains were grown on PDA at 28°C. Seven days old sporulating cultures on PDA served as the starting material for culturing the fungi as described by Gyllang *et al.* (1977). For each strain, a loopful of material taken from the seven-day old sporulating culture on PDA was inoculated in a tissue culture flask containing 225 ml of Peptone, Yeast Extract and Dextrose medium. The culture was grown for 3 weeks at 20°C. After the cultivation period the

entire culture was homogenized by vigorously shaking the content of the tissue culture flask.

5. Analysis of activation of the spores in the culture homogenate. Activated spores were defined as described in the current patent application as "being significantly more swollen than the dormant size, the size of the spores being increased by a factor preferably between 1.2 and 10 over the dormant spore size and/or having one or more germ tubes per spore."

One of the first steps of activation is, indeed, uptake of water, and this is reflected by an increase in volume of the spore by swelling and/or by formation of one or more germ tubes.

Three different samples of 10 μL of each culture homogenate were examined microscopically at 500X magnification (Jenaval, Hainaut, Belgium). Approximately 10 microscopic fields per sample were evaluated. To increase the amount of spores per microscopic field, the culture homogenates were concentrated by centrifugation (Hettich EBA 12, Tuttlingen, Germany; 3000 g, 5 min.) prior to microscopic evaluation.

Photomicrographs of all the microscopic fields were taken and the size of the spores was measured on the printouts of these images. This is in contrast to the method used in the experiments described in the previous report, where the spore size was evaluated directly by microscopic analysis.

Images of the microscopic fields were captured by means of a JVC (TK-C 1381) digital color video camera and saved as a windows bitmap (24 bit) using miro Television software. The image of a microscopic grid (WILD, Heerbrugg, Switzerland; 0.01 mm; 0.10 mm) was captured at the same magnification as was used to capture the images of the spores. This allowed to calculate the

magnification factor and the actual spore size from the measured size on the printouts.

All images were printed in the same manner (full scale) and the size of the spores not occurring in flocs or pairs and that were not attached to mycelium fragments was measured on the printouts. Each spore was measured in the same manner: the largest diameter was measured including the edges of the spore. The actual spore size was calculated by dividing the measured spore size on the printout by the magnification. Also, the absence or presence of germ tubes was recorded for each spore. All observed spores were divided in classes according to size, each class spanning a range of 0.8μm. This gives five size classes for *R. oryzae*, three for *A. fumigatus*, and four for *A. amstelodami*. This allows us to detect any shift in spore size that would occur between incubation time 0 hours and incubation time 6 hours, and that would be indicative of spore activation.

Results

6. Analysis of spore activation. The size of various dormant fungal spores is described by Pitt and Hocking (1997). According to this reference, the sporangiophores of *Rhizopus oryzae* are of variable shape, ellipsoidal to broadly fusiform or irregularly angular, commonly 5.0-8.0 μm long, the condiospores of *Aspergillus amstelodami* are spherical to subspheroidal with 4.0-5.0 μm diameter; the condiospores of *Aspergillus fumigatus* are spherical to subspheroidal with 2.5-3.0 μm diameter. Our own observations of dormant spores of the three tested strains according to the method described above were as follows: the sporangiospores of *Rhizopus oryzae* were 3.9-7.8 μm long; the

condiospores of *Aspergillus amstelodami* had a diameter of 3.1-6.3 μm; the condiospores of *Aspergillus fumigatus* had a diameter of 2.3-4.7 μm. Pictures of the microscopic grid and of the spores at 0 hour, 6 hours at 20°C and 6 hours at 42°C are shown in pictures 1 to 10.

The results of the evaluation of the spore size in the culture homogenates at time 0 hours and after 6 hours incubation at 20°C or at 42°C for *Rhizopus oryzae*, *Aspergillus amstelodami* and *Aspergillus fumigatus* are shown in Tables I, II and III respectively.

Table I - Size Distribution of Spores Occurring in Culture Homogenates of *Rhizopus Oryzae*

Incubation time - 0 h.						
Size Range[a] (μm)	Number of Spores			% of the Total Number of Analyzed Spores		
	1[b]	2[b]	3[b]	1[b]	2[b]	3[b]
3.9 - 4.7	24	54	31	16	23	18
4.7 - 5.5	67	125	99	46	54	59
5.5 - 6.3	55	48	38	37	21	22
6.3 - 7.0	1	2	1	1	1	1
7.0 - 7.8	-	1	-	0	1	0
Total No. of Spores	147	230	169			

[a] The size of a spore in a certain range is larger than the lower limit and smaller than or equal to the upper limit.

[b] Three samples of 10 μl of spore suspension analyzed for each incubation condition.

Incubation time - 6 h., 20°C						
Size Range[a] (μm)	Number of Spores			% of the Total Number of Analyzed Spores		
	1[b]	2[b]	3[b]	1[b]	2[b]	3[b]
3.9 - 4.7	43	74	35	26	43	24
4.7 - 5.5	87	72	67	52	42	45
5.5 - 6.3	32	26	45	19	15	30
6.3 - 7.0	3	-	2	2	0	1
7.0 - 7.8	1	-	-	1	0	0
Total No. of Spores	166	100	172			

[a] The size of a spore in a certain range is larger than the lower limit and smaller than or equal to the upper limit.

[b] Three samples of 10 μl of spore suspension analyzed for each incubation condition.

Incubation time - 6 h., 42°C						
Size Range[a] (μm)	Number of Spores			% of the Total Number of Analyzed Spores		
	1[b]	2[b]	3[b]	1[b]	2[b]	3[b]
3.9 - 4.7	30	19	20	10	14	20
4.7 - 5.5	105	58	54	36	41	53
5.5 - 6.3	142	62	26	49	44	25
6.3 - 7.0	10	1	2	4	1	2
7.0 - 7.8	2	-	-	1	0	0
Total No. of Spores	289	140	102			

[a] The size of a spore in a certain range is larger than the lower limit and smaller than or equal to the upper limit.

[b] Three samples of 10 μl of spore suspension analyzed for each incubation condition.

Table II - Size Distribution of Spores Occurring in Culture Homogenates of *Aspergillus fumigatus*

Time - 0						
Range[a] (μm)	Number of Spores			% of the Total Number of Spores		
	1	2	3	1	2	3
2.3 - 3.1	27	20	22	22	18	16
3.1 - 3.9	67	71	79	56	64	58
3.9 - 4.7	26	20	35	22	18	26
Total No. of Spores	120	111	136			

[a] The size of a spore in a certain range is larger than the lower limit and smaller than or equal to the upper limit.

Time - 6 h., 20°C						
Range[a] (μm)	Number of Spores			% of the Total Number of Spores		
	1	2	3	1	2	3
2.3 - 3.1	10	8	7	9	6	6
3.1 - 3.9	65	112	66	61	89	59
3.9 - 4.7	32	6	39	30	5	35
Total No. of Spores	107	126	112			

[a] The size of a spore in a certain range is larger than the lower limit and smaller than or equal to the upper limit.

Time - 6 h., 42°C						
Range[a] (μm)	Number of Spores			% of the Total Number of Spores		
	1	2	3	1	2	3
2.3 - 3.1	6	3	11	4	3	8
3.1 - 3.9	84	68	96	56	72	66
3.9 - 4.7	61	24	39	40	25	26
Total No. of Spores	151	95	148			

[a] The size of a spore in a certain range is larger than the lower limit and smaller than or equal to the upper limit.

Table III - Size Distribution of Spores Occurring in Culture Homogenates of *Aspergillus amstelodami*

Time - 0						
Range[a] (μm)	Number of Spores			% of the Total Number of Spores		
	1	2	3	1	2	3
3.1 - 3.9	10	4	7	10	4	6
3.9 - 4.7	52	54	64	51	52	56
4.7 - 5.5	30	34	31	29	32	27
5.5 - 6.3	10	13	13	10	12	11
Total No. of Spores	102	105	115			

[a] The size of a spore in a certain range is larger than the lower limit and smaller than or equal to the upper limit.

Time - 6 h., 20°C						
Range[a] (μm)	Number of Spores			% of the Total Number of Spores		
	1	2	3	1	2	3
3.1 - 3.9	2	-	2	3	0	5
3.9 - 4.7	44	17	21	67	41	51
4.7 - 5.5	14	19	15	21	45	37
5.5 - 6.3	6	6	3	9	14	7
Total No. of Spores	66	42	41			

[a] The size of a spore in a certain range is larger than the lower limit and smaller than or equal to the upper limit.

Time - 6 h., 42°C						
Range[a] (μm)	Number of Spores			% of the Total Number of Spores		
	1	2	3	1	2	3
3.1 - 3.9	2	-	-	3	0	0
3.9 - 4.7	37	32	37	61	73	53
4.7 - 5.5	17	10	21	28	23	30
5.5 - 6.3	5	2	12	8	4	17
Total No. of Spores	61	44	70			

[a] The size of a spore in a certain range is larger than the lower limit and smaller than or equal to the upper limit.

7. As set forth in more detail below, spore activation is not a process that occurs quickly and if spore activation occurred at 0 hours, one would see further activation at least after 3 hours, and would certainly see further activation after 6 hours.

8. In contrast, treatment of *Rhizopus oryzae* ATCC 9363 spores as described in the current patent application resulted in a high level of activation of the spores as more than 90% of the spores had a size of more than 9.4 μm and/or had one or more germ tubes per spore, hence, providing further evidence of non-activation of spores as described in the present data.

Statistical Analysis

9. The results from the data reported in this declaration show no statistical increase to a larger spore size. This means that, for none of the three fungi studied, the spores increase in size when spore suspensions are prepared according to Gyllang et al. In addition, none of the observed spores had developed a germ tube.

10. For my statistical conclusion, for each size range within the size distribution of spores of a fungus, the average result of the three analyzed samples and the standard deviation were calculated.

11. The size distribution of spores and standard deviation for each size range for 0 hour, 6 hours at 20°C and 6 hours at 42°C are shown in Table IV, a-c; Table V, a-c; and Table VI, a-c for *Rhizopus oryzae*, *Aspergillus fumigatus*, and *Aspergillus amstelodami*, respectively.

12. In Figures 1, 3 and 5, shown below, the average result and standard deviation for each size range of the size distribution of the spores at incubation time - 0 hour and at incubation time - 6 hours, 20°C are presented, while Figures 2, 4 and 6, show the average result and standard deviations for each

size range of the size distribution of the spores at incubation time - 0 hour and at incubation time - 6 hours, 42°C.

13. In order to compare the results obtained for a certain size range of the size distribution of spores at incubation time - 0 hour with the results obtained for the corresponding size range of the size distribution of spores at incubation time - 6 hours, 20°C and to compare the results obtained for a certain size range of the size distribution of spores at incubation time - 0 hour with the results obtained for the corresponding size range of the size distribution of spores at incubation time - 6 hours, 42°C, a T-test (5% level of significance, two-tailed distribution, two-sample unequal variance) was performed.

Table IVa. Size distribution of spores occurring in culture homogenates of *Rhizopus oryzae*. a. incubation time - 0 h.

size range[a] (µm)	% of the total number of analyzed spores			average	St dev.
	1[b]	2[b]	3[b]		
3,9-4,7	16	23	18	19	4 - 3.6
4,7-5,5	46	54	59	53	7
5,5-6,3	37	21	22	27	9
6,3-7,0	1	1	1	1	0
7,0-7,8	0	1	0	0	1

[a] The size of a spore in a certain range is larger than the lower limit and smaller than or equal to the upper limit
[b] Three samples of 10 µl of spore suspension analyzed for each incubation condition

Table IVb. Size distribution of spores occurring in culture homogenates of *Rhizopus oryzae*. b. incubation time - 6 h, 20°C

size range[a] (µm)	% of the total number of analyzed spores			average	St dev.
	1[b]	2[b]	3[b]		
3,9-4,7	26	43	24	31	10.4
4,7-5,5	52	42	45	46.3	5
5,5-6,3	19	15	30	22	8
6,3-7,0	2	0	1	1	1
7,0-7,8	1	0	0	0	0

[a] The size of a spore in a certain range is larger than the lower limit and smaller than or equal to the upper limit
[b] Three samples of 10 µl of spore suspension analyzed for each incubation condition

Table IVc. Size distribution of spores occurring in culture homogenates of *Rhizopus oryzae*. b. incubation time - 6 h, 42°C

size range[a] (µm)	% of the total number of analyzed spores			average	St dev.
	1[b]	2[b]	3[b]		
3,9-4,7	10	14	20	15	5
4,7-5,5	36	41	53	44	9
5,5-6,3	49	44	25	40	12
6,3-7,0	4	1	2	2	2
7,0-7,8	1	0	0	0	0

[a] The size of a spore in a certain range is larger than the lower limit and smaller than or equal to the upper limit
[b] Three samples of 10 µl of spore suspension analyzed for each incubation condition

Table IVd

Results of T-test (5% level of significance, two-tailed distribution, two-sample unequal variance)

size range[a] (µm)	p-value	
	0h – 6h, 20°C	0h – 6h, 42°C
3,9-4,7	0,19	0,23
4,7-5,5	0,27	0,22
5,5-6,3	0,48	0,23
6,3-7,0	0,60	0,25
7,0-7,8	0,75	0,81

Table Va. Size distribution of spores occurring in culture homogenates of *Aspergillus fumigatus*. a. incubation time - 0 h.

size range[a] (µm)	% of the total number of analyzed spores			average	St dev.
	1[b]	2[b]	3[b]		
2,3-3,1	22	18	16	19	3
3,1-3,9	56	64	58	59	4
3,9-4,7	22	18	26	22	4

[a] The size of a spore in a certain range is larger than the lower limit and smaller than or equal to the upper limit
[b] Three samples of 10 µl of spore suspension analyzed for each incubation condition

Table IIb. Size distribution of spores occurring in culture homogenates of *Aspergillus fumigatus*. b. incubation time – 6 h, 20°C

size range[a] (µm)	% of the total number of analyzed spores			average	St dev.
	1[b]	2[b]	3[b]		
2,3-3,1	9	6	6	7	2
3,1-3,9	61	89	59	70	17
3,9-4,7	30	5	35	23	16

[a] The size of a spore in a certain range is larger than the lower limit and smaller than or equal to the upper limit
[b] Three samples of 10 µl of spore suspension analyzed for each incubation condition

Table IIc. Size distribution of spores occurring in culture homogenates of *Aspergillus fumigatus*. b. incubation time – 6 h, 42°C

size range[a] (µm)	% of the total number of analyzed spores			average	St dev.
	1[b]	2[b]	3[b]		
2,3-3,1	4	3	8	5	3
3,1-3,9	56	72	66	64	8
3,9-4,7	40	25	26	31	8

[a] The size of a spore in a certain range is larger than the lower limit and smaller than or equal to the upper limit
[b] Three samples of 10 µl of spore suspension analyzed for each incubation condition

Results of T-test (5% level of significance, two-tailed distribution, two-sample unequal variance)

size range[a] (µm)	p-value	
	0h – 6h, 20°C	0h – 6h, 42°C
2,3-3,1	0,01	0,01
3,1-3,9	0,40	0,40
3,9-4,7	0,90	0,20

Table IIIa. Size distribution of spores occurring in culture homogenates of *Aspergillus amstelodami*. a. incubation time – 0 h.

size range[a] (µm)	% of the total number of analyzed spores			average	St dev.
	1[b]	2[b]	3[b]		
3,1-3,9	10	4	6	7	3
3,9-4,7	51	52	56	53	2
4,7-5,5	29	32	27	30	3
5,5-6,3	10	12	11	11	1

[a] The size of a spore in a certain range is larger than the lower limit and smaller than or equal to the upper limit
[b] Three samples of 10 µl of spore suspension analyzed for each incubation condition

Table IIIb. Size distribution of spores occurring in culture homogenates of *Aspergillus amstelodami*. b. incubation time – 6 h, 20°C.

size range[a] (µm)	% of the total number of analyzed spores			average	St dev.
	1[b]	2[b]	3[b]		
3,1-3,9	3	0	5	3	2
3,9-4,7	67	41	51	53	13
4,7-5,5	21	45	37	34	12
5,5-6,3	9	14	7	10	4

[a] The size of a spore in a certain range is larger than the lower limit and smaller than or equal to the upper limit
[b] Three samples of 10 µl of spore suspension analyzed for each incubation condition

Table IIIc. Size distribution of spores occurring in culture homogenates of *Aspergillus amstelodami*. b. incubation time – 6 h, 42°C

size range[a] (µm)	% of the total number of analyzed spores			average	St dev.
	1[b]	2[b]	3[b]		
3,1-3,9	3	0	0	1	2
3,9-4,7	61	73	53	62	10
4,7-5,5	28	23	30	27	4
5,5-6,3	8	4	17	10	7

[a] The size of a spore in a certain range is larger than the lower limit and smaller than or equal to the upper limit
[b] Three samples of 10 µl of spore suspension analyzed for each incubation condition

Results of T-test (5% level of significance, two-tailed distribution, two-sample unequal variance)

size range[a] (µm)	p-value	
	0h – 6h, 20°C	0h – 6h, 42°C
3,1-3,9	0,16	0,07
3,9-4,7	0,99	0,25
4,7-5,5	0,57	0,37
5,5-6,3	0,71	0,76

14. The results of the T-tests for each of the spores at 0 hour, 6 hours at 20°C and 6 hours at 42°C are shown in Tables IVd, Vd and VId.

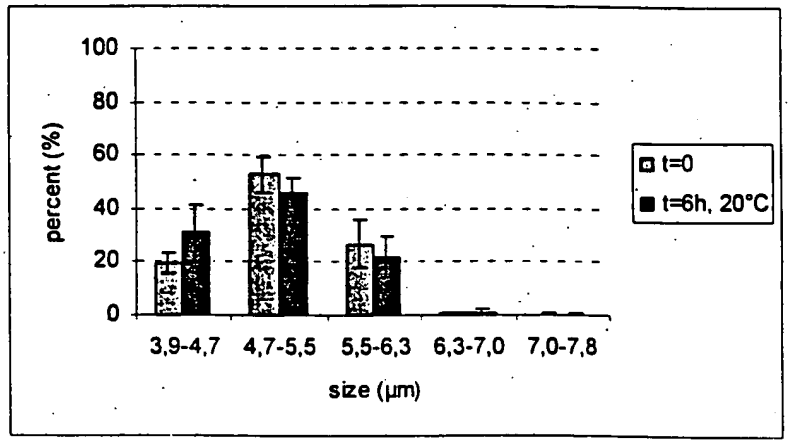

Figure 1. Size distribution of spores occurring in culture homogenates of *Rhizopus oryzae*. (a.) incubation time - 0h and (b.) incubation time - 6h, 20°C; Average results and standard deviation are presented.

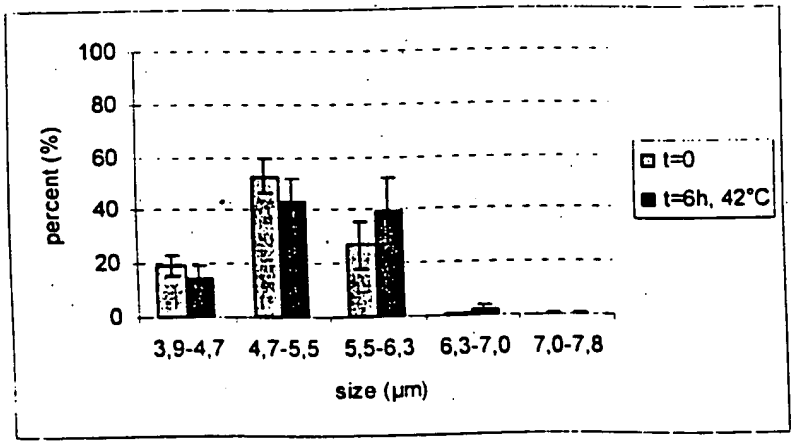

Figure 2. Size distribution of spores occurring in culture homogenates of *Rhizopus oryzae*. (a.) incubation time - 0h and (b.) incubation time - 6h, 42°C; Average results and standard deviation are presented.

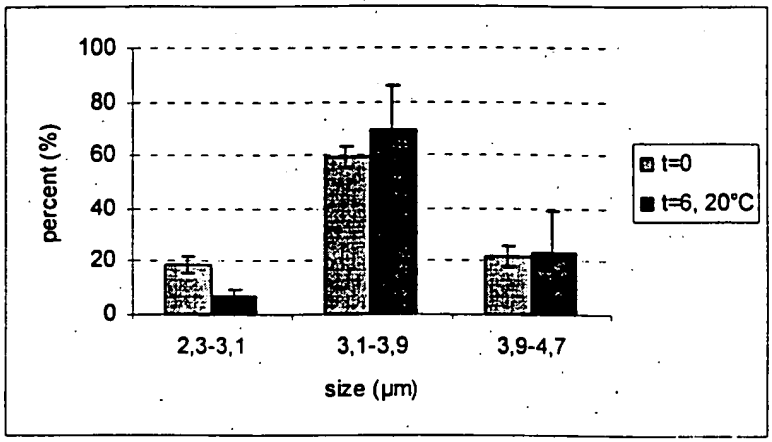

Figure 3. Size distribution of spores occurring in culture homogenates of *Aspergillus fumigatus*. (a.) incubation time – 0h and (b.) incubation time – 6 h, 20°C; Average results and standard deviations are presented.

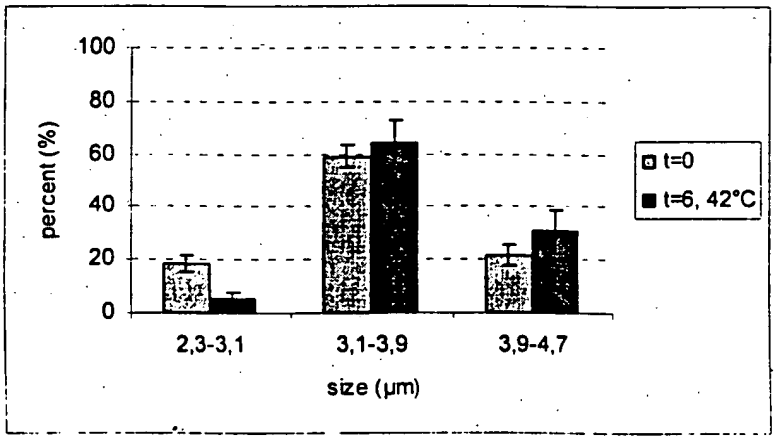

Figure 4. Size distribution of spores occurring in culture homogenates of *Aspergillus fumigatus*. (a.) incubation time – 0h and (b.) incubation time – 6 h, 42°C; Average results and standard deviations are presented.

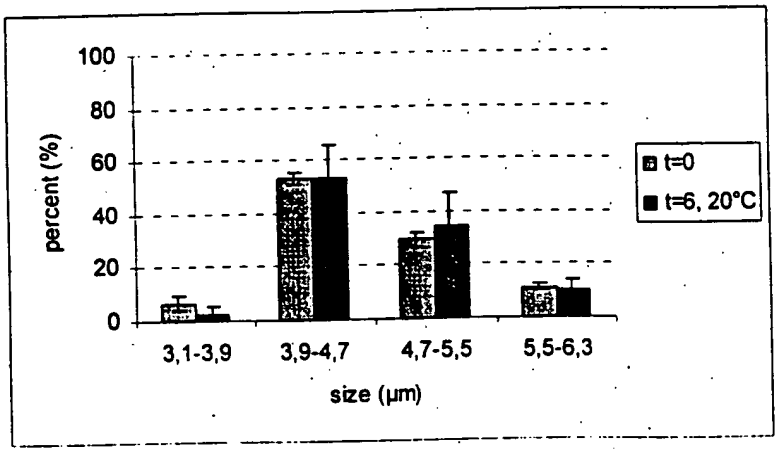

Figure 5. Size distribution of spores occurring in culture homogenates of *Aspergillus amstelodami*. (a.) incubation time – 0h and (b.) incubation time – 6 h, 20°C; Average results and standard deviations are presented.

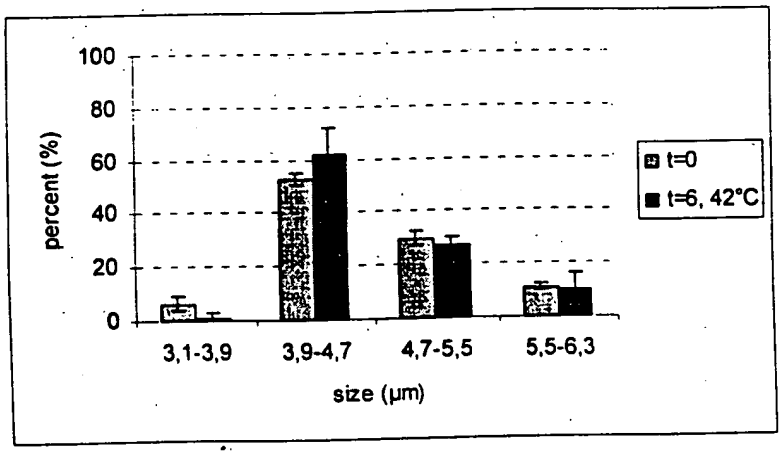

Figure 6. Size distribution of spores occurring in culture homogenates of *Aspergillus amstelodami*. (a.) incubation time – 0h and (b.) incubation time – 6 h, 42°C; Average results and standard deviations are presented.

15. No statistical increase was found for any of the size ranges of the size distribution of each fungus. The lower size range (2.3 - 3.1μ) of *Aspergillus fumigatus* shows a statistical decrease, however, the statistically significant decrease in the percent of total number of analyzed spores in this range did not result in a statistically significant increase in the percent of the total number of analyzed spores in a higher size range (3.1 - 3.9μm and 3.9 - 4.7μm).

16. Because there is no statistical indication of an increase in spore size for any of the spores studied, the results show that there is no indication of spore activation for *R. oryzae*, *A. amstelodami* and *A. fumigatus* in spore suspensions as prepared according to Gyllang, even if the spore suspensions are given an additional incubation of 6 hours, a step that is not specified by Gyllang. Further, it cannot be concluded that the small decrease in spore size observed in *Aspergillus fumigatus* after 6 hours is an indication of spore activation, because it did not result in a statistically significant increase in spore size. In addition, this result is obtained only after an incubation step of 6 hrs, a step that is not specified in the Gyllang procedure.

17. Several examples in the literature show that spore swelling as a result of spore activation is a slow process. Depending on the organism and activation conditions, the onset of activation has been reported to occur after 1 hour (see Ekundayo and Carlile, 1964, and Fig. 1 therein) or after 2.5 to 3.5 hours (see Medwid et al., 1984, and Fig. 3 therein). In the same studies, swelling of the spores continued at least until respectively 8 hours and 4 hours of incubation before reaching a

maximum. Consequently, we are correct in accepting the spore size measured immediately (i.e., within minutes) after homogenization of the spores in the growth medium, as the dormant spore size, and to evaluate spore activation by the increase in spore size in a time frame between 0 and 6 hours after homogenization. This is especially the case where there was no statistical increase in spore size after 6 hours at 20°C and 42°C and no germination tubes were observed after such time at such temperatures.

18. The data generated by the experiment described herein justifies my conclusion that the spore size distribution at incubation time - 6 hours, 20°C, and incubation time - 6 hours, 42°C for the three fungi tested is not statistically significantly different from the spore size distribution at incubation time - 0 hour and that the spores did not significantly increase in size upon incubation for 6 hours either at 20°C or 42°C. From this data, it should also be concluded that the spores prepared according to the Gyllang procedure, i.e., after 0 hours of incubation are not activated.

19. Together with the examples in my patent application, this experiment illustrates that successful activation depends on incubation of dormant spores for a sufficient time at a suitable temperature and in a suitable medium. In the spore suspension as prepared by Gyllang *et al.* (1977), the medium is an exhausted growth medium that does not provide the suitable conditions for spore activation, and the spores are not incubated for a sufficient time at a sufficient temperature. As can be seen from the references attached hereto (Medwid et al., 1984 and Ekundayo et al., 1964), prior to the filing of the above patent

application, it was known how to activate dormant spores and from the general literature existing at the time the above-identified instant patent application was filed, one would know how to activate dormant spores, especially after reading the specification of the instant application.

20. Abbreviations used. PDA, Potato Dextrose Agar; ATCC, American Type Culture Collection; CBS, Centraalbureau voor Schimmelcultures; VTT, Technical Research Centre of Finland; Ac, activated.

21. References.

- Ekundayo et al., *The Germination of Sporangiospores Rhizopus arrhizus; Spore Swelling and Germ - Tube Emergence*, J. Gen. Microbiol. (1964) 35, 261-269.
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- Kaiser, C., Michaelis, S. and Michell, A., *Methods in yeast genetics*, Appendix A, p. 207, Cold Spring Harbor Laboratory Press, New York, USA, 1994.
- Medwid et al., *Germination of Rhizopus oligoporus Sporangiospores*, Applied and Environmental Microbiology, Dec. 1984, p. 1067-1071.
- Pitt, J.I. and Hocking, A.D. Fungi and food spoilage, second edition, Blackie Academic & Professional, London, UK, 1997.

22. Addendum. The printed photomicrographs (40% relative to the original image size) of sample 1 of each experimental

condition are included in Pictures 2 to 10. The printed photomicrograph (40% relative to the original image size) of the microscopic grid is included in Picture 1. Al original images are included in digital format (CD-ROM).

Picture 1	microscopic grid;
Picture 2	*Rhizopus oryzae* ATCC 9363, spores in the culture homogenate at time 0 (sample 1);
Picture 3	*Rhizopus oryzae* ATCC 9363, spores in the culture homogenate after 6 hours incubation at 20°C (sample 1);
Picture 4	*Rhizopus oryzae* ATCC 9363, spores in the culture homogenate after 6 hours incubation at 42°C (sample 1);
Picture 5	*Aspergillus fumigatus* CBS 148.89, spores in the culture homogenate at time 0 (sample 1);
Picture 6	*Aspergillus fumigatus* CBS 148,89, spores in the culture homogenate after 6 hours incubation at 20°C (sample 1);
Picture 7	*Aspergillus fumigatus* CBS 148.89, spores in the culture homogenate after 6 hours incubation at 42°C (sample 1);
Picture 8	*Aspergillus amstelodami* VTT D-76035, spores in the culture homogenate at time 0 (sample 1);
Picture 9	*Aspergillus amstelodami* VTT D-76035, spores in the culture homogenate after 6 hours incubation at 20°C (sample 1);

Picture 10 *Aspergillus amstelodami* VTT D-76035, spores in the culture homogenate after 6 hours incubation at 42°C (sample 1).

The undersigned, being warned that willful false statements and the like are punishable by fine or imprisonment, or both (18 U.S.C. §1001) and may jeopardize the validity of the application or any patent issuing thereon, hereby declares that the above statements made of my own knowledge are true and that all statements made on information and belief are believed to be true.

Date: Herent 01/02/01

Theo Coppens